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由 *Peronospora belbahrii* 引起之羅勒露菌病

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摘 要

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西元 2009 年 3 月在南投埔里及雲林虎尾地區之羅勒植株上發現一種新病害，其病徵爲葉片黃化及輕微皺縮，在高濕的環境下葉背易產生黑褐色絨毛狀物，經鏡檢爲一種露菌之孢囊柄及孢囊。病原菌孢囊柄呈現透明及二至五次分叉，長度 223.1-608.9 μm (平均403.6 μm)，其頂端著生黑褐色卵圓形孢囊，大小 27.3-35.4×19.8-27.7 μm (平均 32.2×22.5 μm)，長寬比 1.1-1.8 (平均 1.4)，自葉片取下孢囊製成孢囊懸浮液噴灑於羅勒葉背進行人工接種，8天後可造成與田間相同之病徵，鏡檢罹病組織觀察到與接種相同之孢囊，完成柯霍氏法則 (Koch's postulates)，證實該菌對羅勒具有病原性，但不會感染薄荷、白菜、葉萵苣及甜瓜。依據其形態特徵、寄主範圍測試及 ITS 增幅序列分析比對，鑑定此菌爲 *Peronospora belbahrii* Thines，此菌所引起之羅勒露菌病爲臺灣首次之報導。

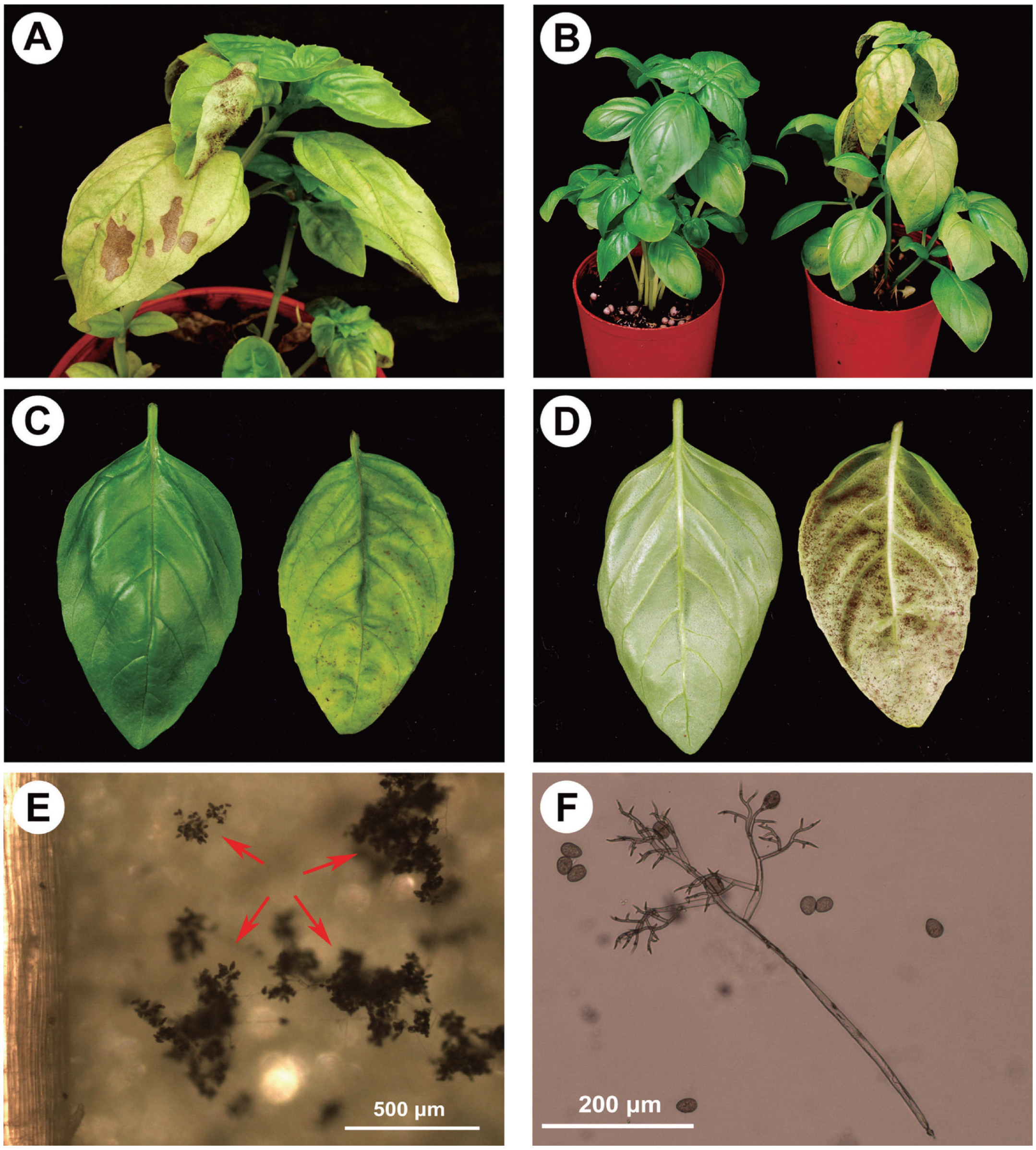
關鍵詞：羅勒、露菌病

羅勒 (英名: basil, common basil, sweet basil，學名: *Ocimum basilicum* L.) 爲唇形花科 (Labiatae) 羅勒屬 (*Ocimum*) 的植物，熱帶至溫帶地區都有分佈與栽培。羅勒之栽培品種眾多且應用範圍廣泛，包括做爲芳香香料、食物烹調及美容保養等許多用途。在歐美羅勒主要用來提煉或萃取精油及沖泡飲用，而在臺灣則主要作爲食物香辛調味料 [10]。臺灣常見的品種一爲全株呈淡綠色，俗稱白骨九層塔或青骨九層塔 (青莖種)，另一種爲植株莖及葉脈部份呈紫色，俗稱紅骨九層塔 (紫莖種)。近年來香草植物栽培盛行，許多歐美香草類羅勒品種被廣泛引進臺灣栽植，例如甜羅勒 (sweet basil)、紫羅勒 (purple ruffles basil)、香球羅勒 (spicy globe basil)、檸檬羅勒 (lemon basil)、桂皮羅勒 (cinnamon basil) 等，爲同種之不同栽培品種，除食用及提煉精油之外，部分品種亦具有觀賞價值 [10, 15]。

在 2009 年 3 月，於南投埔里及雲林虎尾等羅勒栽培區陸續發現甜羅勒、紫羅勒及白骨九層塔等品種感染一種以前未曾記載之新病害。在感染初期，植株葉片產生黃化及輕微皺縮扭曲的病徵 (圖一、A)，黃化區域初受葉脈侷限，隨後在其背面產生黑褐色絨毛狀物 (圖一、E)，病斑亦逐漸擴大，並由黃化病徵轉爲褐色壞疽病徵，末期葉片壞疽萎凋，在秋末至隔年春初，低溫高濕之氣候時大面積發生並快速蔓延。顯微鏡下觀察可發現黑褐色絨毛狀物，爲一種露菌之孢囊柄及孢囊 (圖一、E)。孢囊柄呈現透明及二至五次分叉 (圖一、F)，長度 223.1-608.9 μm (平均 403.6 μm)，其頂端著生黑褐色卵圓形孢囊 (圖一、F)，大小 27.3-35.4×19.8-27.7 μm (平均 32.2×22.5 μm)，長寬比 1.1-1.8 (平均 1.4)。以無菌水輕輕沖洗下葉背之孢囊，並以無菌水調整孢囊懸浮液濃度至每毫升約 1×10^6 個孢囊，分別取 100 μl 以消毒過之三角玻棒均勻塗佈於 2 % 水瓊脂培養基平板 (Bacto^{TM} agar, Becton, Dickinson and Company, USA) 及 4 % 馬鈴薯瓊脂培養基平板 (Potato dextrose agar, Difco Laboratory, Detroit, MI, USA)，每天以 24±2℃，太陽燈管 (Sun-Light, FL-10D, China Electric Mfg. Co., Taiwan) 光照 12 小時，7 天後

檢視並無菌落生長。以無菌水洗下罹病葉背上之孢囊後，分別置於 4±2℃ 及 24±2℃ 定溫箱中，於 16 及 24 小時後在顯微鏡下觀察均無游走孢子產生，依據上述特徵初步鑑定該露菌為 *Peronospora* sp.[1]。

以無菌水由罹病葉背輕輕沖洗下孢囊，並以無菌水調整孢囊懸浮液濃度至每毫升約 1×10^{6} 個孢囊，噴霧接種到健康甜羅勒植株葉背 (株齡約 6 週之甜羅勒，種植於盛有泥炭土的 5 吋軟盆)，以乾淨塑膠袋套袋保持密封狀態並置於定溫箱中，每天以 22±2℃，太陽燈管 (Sun-Light, FL-10D, China Electric Mfg. Co., Taiwan) 光照 10 小時，18±2℃ 保持黑暗 14 小時，接種後第 3 天打開套袋，接種後第8天發現甜羅勒葉片出現黃化及輕微皺縮病徵 (圖一、B, C)，第 11 天於葉背出現黑褐色絨毛狀物 (圖一、D)，經顯微鏡鏡檢與田間病葉上之露菌相同，證實該菌對甜羅勒具病原性，而在相同條件下接種到健康之薄荷 (Coleus, *Mentha spicata* L.)、



圖一、羅勒感染露菌病之病徵與病原菌。A：葉片黃化及皺縮扭曲之病徵。B：對照健康植株 (左) 與接種後出現病徵的人工接種甜羅勒 (右)。C：健康甜羅勒葉片 (左) 與病葉 (右)。D：健康 (左) 與病葉背 (右)。E：解剖顯微鏡下病原菌之孢囊柄與孢囊 (箭頭所指處)。F：光學顯微鏡下病原菌之孢囊柄與孢囊。

Fig. 1. The symptoms and the pathogen of the downy mildew of sweet basil. A: The infected plants showed yellow and slight shrink leaves. B: The healthy plants (left) and the artificially inoculated plants (right). C: Leaves of the healthy plant (left) and the disease plant (right). D: The leaves underside of the healthy plant (left) and the disease plant (right). E: The sporangiophores and sporangia (arrows pointed) under stereo-microscope. F: The sporangiophores and sporangia under light-microscope.

白菜 (Pai-tsai, *Brassica chinensis* L.) 圓葉品種、葉萵苣 (Leaf lettuce, *Lactuca sativa* L.) 及甜瓜 (Melon, *Cucumis melo* L.) 植株葉背，至第 21 天仍未產生任何病徵。

根據國外許多關於羅勒露菌病之報導，早在 1933 年便有羅勒感染露菌之記載 [7]，當時鑑定爲薄荷露菌 (*Peronospora lamii* Thines) 所引起 [8]。而自 1990 年以後，該病害便在歐洲 [4, 5, 11]、美洲 [12, 14]、非洲 [13, 18] 等地被廣泛發現並報導，但多認爲該病原菌應不同於薄荷露菌，爾後利用寄主接種試驗及 ITS 序列分析後，進一步將感染羅勒之病原菌定名爲 *P. belbahrii* [2, 17]。本病原菌可附著羅勒種子上傳播 [6]，且該菌之感染與葉片保溼時間及溫度有關，在溫室試驗中持續保濕 6-12 小時病害發生最嚴重，而在 12-27℃ 之間病害均會發生，以 20℃ 時最爲嚴重 [3]，而除了羅勒外，本菌也會感染藿香屬 (*Agastache* sp.) 的植物 [9]。將分別採集自南投埔里之甜羅勒病葉 (菌株編號：P-PL-01，NCBI Accession Number: HM462242)、雲林虎尾之白骨九層塔病葉 (菌株編號：P-HW-01，NCBI Accession Number: HM486901)，及人工接種於甜羅勒上產生 *Peronospora* sp.的病葉 (菌株編號：P-KO-01，NCBI Accession Number: HM462241)，以無菌水洗下罹病葉背之孢囊後所得三個不同菌株，分別以卵菌綱專用之核酸序列 ITS引子對 ITS-O 及 LR [16] 增幅解序，獲得核醣體非轉錄區間 (ITS1+5.8S+ITS2, complete sequence)，再於 NCBI基因資料庫與 *Peronospora belbahrii* (NCBI Accession Number: EU863410.1) 比對其 ITS 序列相同度均達 99% 以上，而與薄荷露菌 (*Peronospora lamii*) ITS 序列 (NCBI Accession Number: AY919303.1) 相同度均爲 87%。依據前述之病原菌型態、寄主範圍測試及 ITS 序列比對分析等各項結果，鑑定所採集之三個菌株爲 *Peronospora belbahrii* Thines，爲該菌在臺灣引起羅勒露菌病之首次報導。

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ABSTRACT

Chen, C. H.[1], Huang, J. H.[1, 3], and Hsieh, T. F.[2] 2010. First report of *Peronospora belbahrii* causing downy mildew on basil. Plant Pathol. Bull. 17: 177-180 ([1] Plant Pathology Division, Agricultural Research Institute, Council of Agriculture, Wufeng, Taichung, Taiwan, R.O.C.; [2] Floriculture Research Center, Agricultural Research Institute, Council of Agriculture, GuKeng, YunLin, Taiwan, R.O.C.; [3] Corresponding author, E-mail: jhhuang@tari.gov.tw; FAX: +886-4-2330-2803)

A new disease of basil (*Ocimum basilicum* L.) was found in the field of Nantu and Yunlin in the spring of 2009. The main symptoms were chlorosis and slight curve of leaves followed by defoliation. The gray, downy molds on the underside of the leaves were observed as the sporangiophores and sporangia. Sporangiophores are hyaline, branched two to five times, 223.1-608.9 μm (avg. 403.6 μm) in length, and ended with sterigmata bearing single sporangia. Sporangia are 27.3-35.4×19.8-27.7 μm (avg. 32.2×22.5 μm) in size, length/width ratio 1.1-1.8 (avg. 1.4), elliptical and grayish in mass. The sporangia were collected from diseased leaves for pathogenicity tests by spore suspension spraying. The pathogen caused chlorosis and shrinkage of leaf on basil as in the field, but didn't cause any symptom on coleus, Pai-tsai, leaf lettuce, and melon. According to the morphology, pathogenicity test, host range, and ITS sequence analysis, the pathogen was identified as *Peronospora belbahrii* Thines. This is the first report of downy mildew of basil caused by *P. belbahrii* in Taiwan.

Keywords: downy mildew, basil, *Peronospora belbahrii*